Effects of Atorvastatin on the growth of *Candida auris* from human isolate

Salma Naqvi[1], Mohannad Khalid Amin Attas[2], Fahad A. Al-Abbasi[2], Firoz Anwar[2], Vikas Kumar[3] Shahnawaz Ahmed[4]
[1]Department of Biomedical Sciences, College of Medicine, Gulf Medical University, Ajman, UAE, [2]Department of Biochemistry, Faculty of Science, King Abdul-Aziz University, Saudi Arabia, [3]NPDD Laboratory, Dept of Pharmaceutical Sciences, Allahabad, India, [4]Jamia Hamdard University, New Delhi, India

ABSTRACT:

Background: The fungal infection, caused by the newly discovered, highly resistant Candida auris, was a major global health threat before COVID-19. The concept of drug repurposing not only addresses the issue of microbial resistance, but also is an easy way to bypass the costly and time-consuming novel drug development. **Rationale**: Statins, which are therapeutically used for the treatment of atherosclerosis, have exhibited some antifungal actions against various fungal spp. Although, no such study was conducted on C. auris. Based on the anti-HMG-CoA reductase activity on ergosterol synthesis, we elucidated the effect of Atorvastatin at clinically administered human dose (0.055g), on Candida auris infection. **Method**: C. auris was isolated from an adult male diabetic patient, and identified on the Vitek system, confirmed on MALDI-TOF MS with 99.9% accuracy. **Observation**: Initially, no growth was seen in the first twenty-four hours, but an unexpected growth was observed after 48 hours, and the colonization further doubled in 96 hours. **Conclusion**: Our investigation provides an alarming awareness to the patients on Atorvastatin- therapy. They should be highly cautious about C. auris, and must take appropriate measures to prevent the infection.

Keywords: *Candida auris*, statins, Atorvastatin, fungal infections

INTRODUCTION

- Multiple drug resistance, rapid global emergence, and high mortality rate of C. auris makes it a pathogen of high consideration by medical professionals and research communities. Genetic reports reveal that this pathogen species emerged simultaneously in different continents. Among the Gulf countries, cases of C. auris have been reported from UAE, Kuwait, Oman and Saudi Arabia.
- Addressing the issue of resistance to several antifungal drugs, the concept of drug-repositioning has recognized the activities of many non-antifungal drugs. Statins, which are therapeutically used for the treatment of atherosclerosis, have shown numerous pleiotropic activities. Their potential to inhibit HMG-CoA-reductase inherit them a plausible antimicrobial action. Antifungal action of statins is associated with their ability to inhibit the biosynthesis of isoprenoids, including geranyl and farnesyl pyrophosphates. These are the key-factors for the synthesis of ergosterol and other bioprocesses of a fungal cell including G-protein regulation, cellular respiration, mitochondrial function and Ras-like signaling. The reduction in ergosterol levels inhibits the fungal growth. Researchers have reported antifungal action of Atorvastatin in vitro against Aspergillus niger and different Candida spp., including C. albicans, C. glabrata, C. krusei, C. kefyr and C. stellatoidea. All species were sensitive to the drug. However, none of the studies has reported the effect of Atorvastatin on C. auris.

STUDY OBJECTIVES

❖ To elucidate the effect of clinically administered dose of Atorvastatin on the highly pathogenic fungus, C. auris.

MATERIALS AND METHODS

- ***Drugs and chemicals***
- Standard laboratory chemicals were procured by the Department of Biochemistry. Faculty of Sciences, King Abdul Aziz University, Jeddah. Pure Atorvastatin was kindly gifted by Jumjum Pharmaceutical, Jeddah, Saudi Arabia.
- ***C. auris strain***
- The strain of C. auris was isolated from a 68-year-old male diabetic patient admitted to Al Noor specialist Hospital, Mecca, Saudi Arabia. The identification of the isolate was performed on the Vitek system, BioMerieus, France (ID: 1128783-1). The isolated strains were further analyzed and confirmed on MALDI-TOF MS (Bruker Inc., MA, USA) (Isolate: CO DR DA S-1) with 99.9% confirmation result. .
- ***Culture media:*** 24 g of Potato Dextrose Agar was used as solid media for fungal strain, dissolved in 1000ml of water, autoclaved at 1210C, 15 lbs pressure for 20 minutes and cooled. A volume of 100 µL of C. auris inoculum was added to the sterile microplates. Pure Atorvastatin was incorporated into one of the culture plates. The plates were subsequently incubated at 37^{0}C and observed after Day 1, Day 2, Day 3, Day 4.
- ***Dose selection:*** Clinically administered human dose of Atorvastatin (0.055g) was selected for the study.

STUDY DESIGN

- The effect of Atorvastatin was studied on C. *auris*, at the normal human dose, with incubation on a solid media of 24 gm of potato dextrose agar (PDA). One plate served as negative control, and the other was inoculated with 0.055gm of Atorvastatin. The plates were observed for four days.

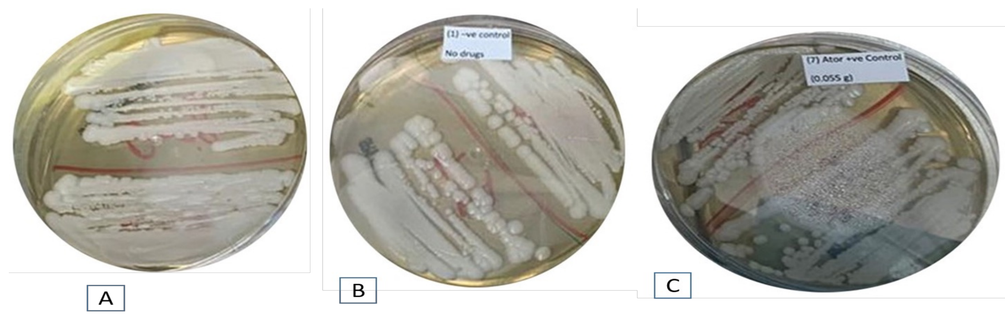

Figure 1. Plates showing growth of C. auris strain A) Master Plate or original C. auris strain; B) Duplicate Master Plate or original C. auris strain; C) C. auris growth in presence of Atorvastatin

Drug concentration	No Drug (Negative control)	Atorvastatin (0.055 g)*
Day 1	-	-
Day 2	++	++
Day 3	++++	++++
Day 4	++++	++++

** Clinically used human dose*

RESULTS

No growth was observed in the negative control as well as the drug treated culture-plates in the first 24 hours. However, an unexpected fungal growth, even in the presence of drug, was observed after 48 hours [Fig 1]. Colonization increased further on day 3 and 4 (Table 1).

DISCUSSION:

❖ Anthropogenic factors help microbes to take global pathways and contribute to rapid resistance to the existing antimicrobial drugs. Similar resistance may have developed in *C auris* since it originated in Japan in the year 2009 to 2021 in Saudi Arabia [1].

❖ Phenotypic plasticity in pathogens often cause alterations in their gene expression and improve microbial survival under hostile conditions. *C. auris* is believed to have a highly plastic genome that gives it a potential to and generate different variants. A change in genetic structure might have produced favorable conditions for this pathogen in presence of Atorvastatin.

❖ A cross-talk between transcriptional factors and chromatin remodeling greatly influences fungal virulence. Scientists have reported the expression of histone proteins during *C. auris* filamentous growth, suggesting that epigenetic mechanisms may alter its genetic pattern. This modified genetic expression may explain its resistance to Atorvastatin [2].

❖ Upregulation of Adenosine binding cassette transporters (ABC proteins) and development of fungal efflux pump results in decreased cellular concentration of the drugs. ABC transporter genes have shown increased expression of clinical isolates of in *C. auris*, thereby decreasing the antifungal activity of Azoles [3].

❖ Change in the target site: Researchers have reported over-expression and site-targeted alteration in *Candida auris*. Mutation in HMG-CoA reductase, the target of Atorvastatin, may facilitate fungal ergosterol synthesis and reduce the drug's efficacy.

❖ Heat shock proteins (Hsp90), an essential protein that regulates the foldings, transportation, maturation and degradation of many other proteins responsible for cell signaling. It can buffer the expression of genetic and epigenetic variations in response to change in the environment of the organism. The new environment for *C. auris* in presence of Atorvastatin may have triggered the excessive synthesis of Hsp90, increasing its drug resistance and enhancing its growth [4].

CONCLUSION

❖ Atorvastatin, although prevents the growth of various Candida spp., but it promotes the growth of *C. auris* from human isolate. To gain insight into how the presence of Atorvastatin promoted the growth of *C. auris*, though, it inhibited the same in other fungal species, future work should aim to decipher the exact mechanism and pathways and disambiguate the experimental observation.

❖ Nonetheless, our investigation provides an awareness that the patients on statin- therapy should be highly cautious about *C. auris* infection, and must take appropriate measures to prevent the infection.

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